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A new principal component analysis-based approach for testing "similarity" of drug dissolution profiles

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ABSTRACT

A new approach for testing batch "similarity" through comparison of drug dissolution profiles, based on principal component analysis with the establishment of a confidence region (PCA-CR), is presented. The dissolution curves corresponding to three brands each of Furosemide and Acetaminophen tablets, taken as model drugs, were prepared by dissolution measurements at multiple pre-specified time points. Reference and test data were simultaneously subjected to PCA and pairwise comparisons between the dissolution characteristics of lots of the same and different brands were carried out. The comparisons involved plotting the weighed scores of the first two principal components of reference and test lots, while decision about "similarity" was made by checking for inclusion of more than 80% of the tablets of the test lot in the 95% confidence ellipse of the reference samples. Two published datasets were also analyzed in the same fashion and all the results were compared with information provided by the difference (f_1) and similarity (f_2) factor tests. Unlike the f_2 criterion, the proposed method reflects variability within the individual dissolution curves, being also highly sensitive to profile (shape and size) variations. Comparison between the area enclosed by the confidence ellipses of the weighed scores plot and the region obtained from the bootstrap-calculated acceptable values of the corresponding f_2 tests suggested that PCA-CR represents, in general, a more discriminating standard.

1. Introduction

In vitro dissolution testing is an economic and useful quality control tool to effectively assure acceptable product quality during different stages of the development and production of tablets, capsules and other solid dosage forms (Dressman and Kramer, 2005). The test enables detection of the influence of key manufacturing factors including excipients, binder and mixing effects, as well as granulation procedure and coating parameters, providing better control of the production process and assuring consistent batch to batch quality of the product. The dissolution has also been employed in product development and during dosage form optimization to assist in proper formulation selection. In addition, it has served as a means to compare different formulations (Naylor et al., 1993) and determine final dissolution specifications for pharmaceutical dosage forms (Elkoshi, 1999).

The dissolution test has also been used during stability studies, helping establish shelf life, and it has been recognized as an important *in vitro* parameter of tablets' quality because of its correlation with drug bioavailability (Williams et al., 1991; Fassihi and Ritschel, 1993; Munday and Fassihi, 1995; Grundy

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et al., 1997). As a result, under certain strictly defined conditions, the test can also be employed as a surrogate of *in vivo* studies for the assessment of product bioequivalence, helping to reduce costs by circumventing the need to perform human volunteer experiments (Leeson, 1995; Yu et al., 1996).

Because it is essential to investigate the drug release characteristics of pharmaceutical preparations, dissolution has become highly significant and one of the primary pharmacopoeial tests that is performed to ensure that tablets, capsules and other drug products comply with pre-established quality standards.

For a drug product, the curve of the mean dissolution rate over time is referred to as its dissolution profile. There are several circumstances under which comparison of the dissolution profiles of two solid oral dosage forms is important. Among them, when an approved formulation is subjected to a post-approval change due to modifications of some critical parameters, including manufacturing site, composition, manufacturing process and batch size. In these cases, FDA guidances for scale-up and post-approval changes for solid oral dosage forms (FDA, 1995) require that the dissolution profiles of the pre-change and post-change products must be "similar".

Another paradigmatic scenario is in the development of generic preparations. Here, a proprietary product, which has been available in the market for some time and has a clinically established efficacy, is selected as a reference against which to compare the new formulation. Because of the "similarity" requirement, the generic preparation should be formulated with its dissolution profile as closely similar as possible to that of the proprietary product.

In response to the need of assessing "similarity", numerous strategies have been proposed for comparing dissolution profiles. These, which are divided in ANOVA-based, model-dependent and model-independent approaches, have been extensively reviewed (Polli et al., 1996, 1997; O'Hara et al., 1998; Costa and Sousa Lobo, 2001). The ANOVA-based methods (Mauger et al., 1986; Yuksel et al., 2000) assume the existence of underlying models, but do not require fitting of a curve. They test statistical differences of the dissolution profiles in terms of "shape" and "size" of the curves, providing probability values related more to statistical equivalence than to pharmaceutical similarity.

Model-dependent methods rely on curve-fitting procedures, which facilitate data analysis and interpretation because they describe the dissolution profiles as functions of a few model parameters that can be determined and statistically compared. In general, however, these are rather rigid representations, there is no universal model to fit all dissolution profiles and there are no established criteria to select the proper mathematical model.

Model-independent methods do not require a preconceived or fitted model. The difference (f_1) and similarity (f_2) factors introduced by Moore and Flanner (1996) as mathematical indices to compare dissolution profiles constitute the most widely known examples of the model-independent approach. This procedure, where the dissolution behaviour of a number of samples (n) of reference (R) and test (T) products are compared at t time points (Eqs. (1) and (2)), is being recommended by the FDA Guidance for Industry (FDA, 1995), and has been accepted by European agencies and other regulatory bodies (Human Medicines Evaluation Unit, 1999). For testing purposes, a discriminatory medium can be identified by varying stirring rate and parameters of the dissolution medium, including pH, ionic strength, volume, etc.

$$f_1 = 100 \left(\frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right) \quad (1)$$

$$f_2 = 50 \log \left\{ \left[\left(1 + \left(\frac{1}{n} \right) \sum_{t=1}^{n} (R_t - T_t)^2 \right) \right]^{-0.5} \times 100 \right\} \quad (2)$$

Since drug release depends on many variables, such as the physicochemical properties of the drug, the excipients and the structural properties of the tablet matrix, an understanding of the complex causalities between different variables and responses becomes difficult. Therefore, for decision taking, it is useful to collapse this complex information into a minimum identifiable number of parameters. As a variable simplification approach, in many cases two batches are compared through the determination of their percentage of dissolved active component at a certain time point. However, this provides less meaningful conclusions than the independent comparison of specifications at each of multiple time points or the analysis of the entire dissolution profile. For such problems, multivariate data analysis is the tool of choice. Multivariate methods such as principal component analysis (PCA) have been suggested for the evaluation of dissolution profiles (Tsong et al., 1997; Adams et al., 2001, 2002), while other approaches including artificial neural networks with similarity factor (Peh et al., 2000; Goh et al., 2002, 2003) and Gaussian mixture models (Lim et al., 2005) as well as partial least squares (Korhonen et al., 2005), have been proposed as multivariate strategies for the prediction of dissolution profiles.

Here, we propose the application of PCA with confidence regions (PCA-CR) as a new and alternative method to compare solid dosage forms dissolution behaviour and decide about their "similarity". The usefulness of the suggested strategy was demonstrated by comparing different brands and lots of tablet preparations containing either Furosemide or Acetaminophen, as models, and also two selected literature datasets. For assessing the scope and limitations of the proposed approach, the PCA-CR results were confronted in each case with the conclusions provided by the corresponding f_1 and f_2 factors, taken as reference.

2. Materials and methods

2.1. Equipment, software and reagents

Dissolution tests were performed with a Hanson SR8-Plus dissolution test station configured as USP-apparatus II (paddle). The amounts of drug dissolved were determined in 1-cm quartz cells, employing a Shimadzu UV-1601PC spectrophotometer interfaced to a computer running UV-Probe software

v. 2.00. Determinations were carried out against a blank of dissolution medium, on filtered samples suitably diluted with dissolution medium, by comparison with standard solutions containing known concentrations of the corresponding analytes. All the reagents employed were of analytical grade; double distilled water was employed as solvent. All the computations were performed in Matlab v. 5.3 (Natwick, MA); the Matlab scripts are freely available from the authors.

2.2. *Tablet preparations and dissolution conditions*

All the brands and lots of Furosemide and Acetaminophen drug products used met the pharmacopoeial specifications for weight variation, content uniformity and assay.

2.2.1. *Furosemide*

Eight lots corresponding to three different brands of tablet products (40 mg) were studied. Each product was randomly labelled with a specific letter for identification, designating with A_1 the reference lot of the innovator product. The release characteristics were determined at $37 \pm 0.5\,^{\circ}C$, using the procedure of the "Dissolution Test 1" of USP 30 (USP Convention, 2007). The medium was 900 ml of Phosphate buffer (0.05 M, pH 5.8) and the stirring rate was 50 rpm. One tablet was used in each vessel, and each test comprised two runs of six tablets yielding a total of 12 tablets per lot (FDA, 1995). During each experiment, aliquots of 3 ml were removed at 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 26 and 30 min, filtered and suitably diluted with medium. The amount of drug dissolved was determined from the absorbances of the samples at 274 nm. Each dissolution curve contained a total of 17 time points.

2.2.2. *Acetaminophen*

Three different brands of tablet products (500 mg) were studied and brand A was used as the reference product (innovator). The other brands were each randomly designated with letters B and C for identification. The dissolutions were determined at $37 \pm 0.5\,^{\circ}C$ in 900 ml of Phosphate buffer (0.05 M, pH 5.8), using a slight modification (stirring rate was 30 rpm) of the USP 30 procedure in order to increase selectivity. One tablet was used in each vessel, and each test comprised two runs of six tablets yielding a total of 12 tablets per lot (FDA, 1995). Aliquots of 3 ml were removed at 2, 6, 10, 14, 18, 22, 26, 30, 45 and 60 min, filtered and suitably diluted with medium. The amount of drug dissolved was determined at 243 nm. Each dissolution curve contained 10 time points.

2.2.3. *Literature data*

Data taken from the following sources were employed: (a) Tsong and Hammerstrom (1994): dissolution curves of three approved batches and a new test batch (12 tablets each, determined at 7 time points). (b) Shah et al. (1998) and Ma et al. (2000): data of a pre-change lot and five post-change lots (12 tablets each, determined at 4 time points).

2.2.4. *Theoretical background of the f_1 and f_2 estimators and the PCA algorithm*

2.2.4.1. Factors f_1 and f_2 as estimators of difference and similarity. Eqs. (1) and (2) correspond to the difference (f_1) and similarity (f_2) factors, respectively (Moore and Flanner, 1996). The f_1 index computes the absolute cumulative differences between drug release in reference and test samples, relative to the drug dissolved in the reference sample. Therefore, the value of this parameter, which is proportional to the average difference between both profiles, depends on which sample is taken as reference. Acceptable values of f_1 are $0 \leq f_1 \leq 15$.

On the other hand, f_2 is a logarithmic function of the reciprocal of the mean square-root transform of the sum of squared errors at all points, and is a measure of the degree of similarity in the percent rate of drug release between two dissolution profiles. The f_2 values are independent from the sample taken as reference, and they range between 0 and 100, with a higher number indicating better similarity between profiles. Acceptable values are $50 \leq f_2 \leq 100$, which is considered equivalent to a difference in approximately 10% between the dissolution profiles being compared (Shah et al., 1998).

2.2.4.2. Principal component analysis. The principles underlying PCA have been extensively discussed elsewhere (Wold et al., 1987); the following is a brief description of this multivariate method.

Given matrix $\mathbf{X}_{(p\times t)}$, where each row contains t different pieces of information gathered from p objects, the column mean centred data matrix **Xc** can be obtained by subtracting the row vector containing the mean values of its columns (**Xm**), from each row of the original matrix (**X**).

In turn, **Xc** can be decomposed into the product of an orthogonal matrix **U**, a diagonal matrix **S** and another orthogonal matrix **V** (Eq. (3)), where $\mathbf{U}\,\mathbf{S}\,\mathbf{V}^{T}$ represents the singular value decomposition (SVD) of **Xc** (Manly, 1986).

$$\mathbf{Xc}_{(p\times t)} = \mathbf{U}_{(p\times t)}\mathbf{S}_{(t\times t)}\mathbf{V}^{T}_{(t\times t)} (p > t) \tag{3}$$

The score matrix $\mathbf{U}_{(p\times t)}$ is the unweighed (normalized) score matrix and represents the projections of the data on the PCs; therefore, similar samples are represented by similar scores. On the other side, the diagonal matrix $\mathbf{S}_{(t\times t)}$ contains the singular values, which are the square roots of the eigenvalues associated to the corresponding PCs (eigenvectors). These diagonal terms reflect the dynamics of the dissolution; therefore, the largest eigenvalues correspond to the dimensions that explain larger amounts of variance of the dataset. Matrix $\mathbf{T}_{(t\times t)}$ known as the weighed (unnormalized) score matrix, is the product between **U** and **S** ($\mathbf{T} = \mathbf{U}\cdot\mathbf{S}$). Finally, the loadings matrix $\mathbf{V}_{(t\times t)}$ contains in its columns the weights contributed by the original variables (eigenvectors) to the PCs.

2.2.5. *Detection of outliers*

Outlier detection was performed by means of Hotelling's test (Jackson, 1991). For that purpose, the test was implemented for each dataset, according to Eq. (4), where μ_x is the mean of the data and $\mathbf{S}_{XX}^{-1}$ is the inverse of the data covariance matrix $\mathbf{S}_{XX}$ (Eq. (5)). The required Mahalanobis distance was calculated according to Eq. (6), where q is the number of dissolution curves in the reference and test lots (Section 2.2.9), and was compared with the corresponding Chi square value at a 99% confidence level and t (number of data points per

curve) degrees of freedom.

$$P[y = (\mathbf{x} - \mu_x)^T \mathbf{S}_{XX}^{-1}(\mathbf{x} - \mu_x) < y_p] = 1 - \alpha \quad (4)$$

$$\mathbf{S}_{XX} = (t - 1)^{-1} \cdot \mathbf{XcXc}^T \quad (5)$$

$$q(\mathbf{x} - \mu_x)^T \mathbf{S}_{XX}^{-1}(\mathbf{x} - \mu_x) < \chi^2_{0.99,t} \quad (6)$$

2.2.6. Selection of the optimum number of principal components

As the principal components (PCs) are weighed in decreasing order of variance coverage, the variation of the dataset can be conveniently expressed in terms of a small number of significant variables, while the remaining variables (residual), contain mostly noise. Thus, the reconstructed data matrix $\mathbf{X}^*c$ can be obtained (Eq. (7)) by employing a reduced number ($r < t$) of PCs.

$$\mathbf{X}^*c_{(p\times t)} = \mathbf{U}^*_{(p\times r)}\mathbf{S}^*_{(r\times r)}\mathbf{V}^{*T}_{(r\times t)} = \mathbf{T}^*_{(p\times r)}\mathbf{V}^{*T}_{(r\times t)},$$
$$[r \le p - 1(p \le t) \text{ and } r \le t(p > t)] \quad (7)$$

The number of significant PCs to be retained (r) can be obtained by different means, including cross-validation, setting a threshold to the minimum variance explained, or evaluating the residuals between $\mathbf{Xc}$ and $\mathbf{X}^*c$. Observation of the shape of the PCs also constitutes a useful hint. In this work, $\mathbf{X}^*c$ was reconstructed from matrices $\mathbf{U}^*$, $\mathbf{S}^*$ and $\mathbf{V}^*$ with an increasing number of PCs, until the optimum number of factors, yielding the error matrix $\mathbf{E}$ (Eq. (8)) similar to instrumental error, was obtained. Two PCs were found to satisfactorily reconstruct the original dataset in the four studied cases.

$$\mathbf{E}_{(p\times t)} = \mathbf{Xc}_{(p\times t)} - \mathbf{X}^*c_{(p\times t)} \quad (8)$$

2.2.7. Confidence regions

In order to test the hypothesis of similarity, the 95% a confidence region ($\alpha = 0.05$) was drawn for each pair of lots being compared, taking into account the variability of the weighed scores data of the reference lot. This confidence ellipse was obtained from Hotelling's test (Eq. (6)) and plotted according to Eq. (9), where d_1 and d_2 are eigenvalues of $\mathbf{S}_{XX}$, while w_1 and w_2 are elements of matrix $w = \mathbf{B}(x - \mu_x)$; the rows of $\mathbf{B}$ are eigenvectors of $\mathbf{S}_{XX}$. w_1 and w_2 provide information related to the orientation of the ellipse, which axes' lengths are defined by $(d_1\chi^2{}_{1-\alpha,r})^{0.5}$ and $(d_2\chi^2{}_{1-\alpha,r})^{0.5}$, respectively. The degrees of freedom (r) of the χ^2 equal the number of the selected PCs (Section 2.2.6).

$$P\left[\left(\frac{w_1^2}{d_1\chi^2_{1-\alpha,r}}\right) + \left(\frac{w_2^2}{d_2\chi^2_{1-\alpha,r}}\right) < 1\right] = 1 - \alpha \quad (9)$$

2.2.8. Bootstrapping procedure for finding the $f_2 \le 50$ region

The mean vector of data $\mathbf{a}_{(1\times t)}$ (dissolution profile) of the reference lot was successively transformed into a new vector $\mathbf{d}_{(1\times t)}$ by replacing some of its items with artificial data containing deviations able to originate f_2 values around 50. This procedure was repeated a number of times, and in each case the values of f_2 and the PCs of the artificial dissolution curve were calculated (Efron and Tibshirani, 1986, 1993; Shah et al., 1998; Adams et al., 2001). Plots of the $f_2 = 50$ ellipses (enclosing the $f_2 \le 50$ region) are shown in the graphics.

2.2.9. Procedure for the comparison of dissolution profiles

Given the data matrices $\mathbf{A}_{(q\times t)}$ and $\mathbf{B}_{(q\times t)}$, containing the dissolution curves of q tablets each corresponding to the lots of dosage forms to be compared, taken at the same t time points, the following five steps are proposed to be sequentially carried out:

(a) Detect outliers in the individual datasets, employing Hotelling's test (Section 2.2.5).
(b) Construct the matrix $\mathbf{X}_{(2q\times t)}$, which contains the data of $\mathbf{A}$ and $\mathbf{B}$ ($2q = p$, see Section 2.2.4.2); mean-center (column-wise) this matrix and carry out the SVD operation on the resulting matrix $\mathbf{Xc}_{(2q\times t)}$ (obtain matrices $\mathbf{U}$, $\mathbf{S}$ and $\mathbf{V}$).
(c) Select the number of PCs to be retained (Section 2.2.6) and compute matrix $\mathbf{T}^*_{(p\times r)}$.
(d) Draw the 95% confidence region (Section 2.2.7), in order to test the hypothesis of similarity.
(e) Decide about "similarity", based on the inclusion of the test data (>80%) in the confidence ellipse of the reference.

3. Results and discussion

3.1. Characteristic features of the proposed PCA-CR approach

The proposed approach for the assessment of "similarity" through the PCA-CR analysis of dissolution curves entails five steps, including (a) detection of outliers in reference and test data matrices; (b) construction and column mean centering of a single data matrix containing both data of reference and test samples, which is submitted to a SVD operation; (c) selection of the number of PCs to be retained; (d) plotting of the weighed scores of reference and test lots, and drawing of the 95% confidence region based on scores plot of the reference lot; (e) "similarity" decision making based on the percentage of test samples included in the above confidence region.

These sequential steps constitute the appropriate means for pre-processing, analyzing and visualizing the data, also establishing a convenient approach for final decision taking. Hotelling's test represents a useful strategy for outlier detection, helping to avoid inclusion of dissolution curves with exceptionally high variability. On the other hand, PCA is a mathematical procedure that allows the representation of a complex set of multivariate data with a reduced number of new and uncorrelated variables (PCs), which are linear combinations of the original data. In the proposed method, joining reference and test datasets and carrying out the SVD on a single matrix allows the optimization of system parameters leading to an improved projection of the test data in the reference–test joint data space; therefore, misadjustements resulting form fitting test data into a pre-established reference model, are avoided.

By discarding feature elements with low variability, PCA allows data visualization and the discovery of hidden trends

in fewer dimensions. The PCs are ordered according to their ability to explain data variability, and in the selected examples discussed below, plot of the weighed scores of the first two PCs is proposed, as these allow satisfactory reconstruction of the original data matrix. This approach is much simpler than that proposed by Tsong et al. (1997), which employs all the PCs for comparison.

After some trial and error experiments, and taking into account that "similarity" is a property of the lot and not of the individual tablets, for decision taking, the following criterion was adopted "test lots are considered to be 'similar' if they contain more than (the arbitrarily chosen value of) 80% of their tablets (Chen and Tsong, 1997) inside of the 95% confidence region of the reference lot". The ">80%" requirement takes into account test data variability, while the 95% confidence ellipse considers variability of the weighed scores in the reference lot.

In order to assess the usefulness of the proposed method, the dissolution curves of Acetaminophen and Furosemide tablets, and two datasets selected from the literature, were individually analyzed by the PCA-CR methodology and compared with the information provided by the f_1/f_2 criteria. Results for each set of data are presented below and discussed separately.

3.2. *Dissolution of Furosemide tablets*

Eight lots of Furosemide tablets, corresponding to three different brands, A (lots A_1, A_2 and A_3), B (lots B_1, B_2 and B_3) and C (lots C_1 and C_2), were studied, with brand A being the innovator. The mean percentages of drug released over a 30-min period are depicted in Fig. 1.

The individual profiles of the eight lots complied with the FDA requirements for the evaluation of similarity and difference; i.e., the tablets dissolved less than 85% of their active principle in the first 15 min, the data coefficient of variation (CV%) was less than 20% for the first time points, being less than 10% for the remaining time points (≥6 min) and the overall CV% was less than 15%. The corresponding f_1 and f_2 values were calculated, employing data acquired at 2, 7, 12, 18 and 26 min, taking care that no more than one time point (26 min) corresponded to more than 85% of dissolved drug. For the sake of the analysis, all of the possible pairwise lot comparisons were carried out, with the results consigned in Table 1.

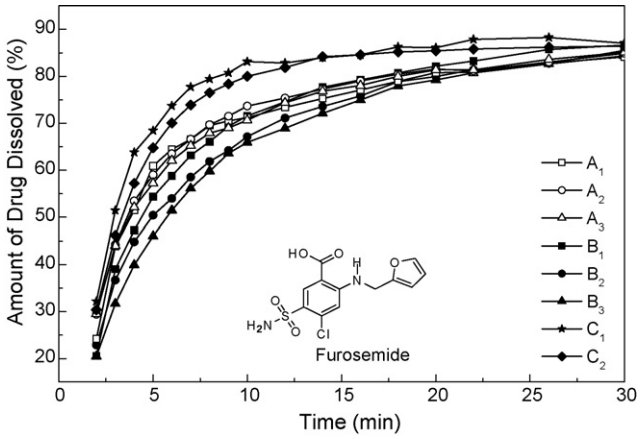

Fig. 1 – Dissolution profiles of eight lots of Furosemide tablets, corresponding to three different brands (A, B and C); for the sake of clarity, error bars (<20% for the first time points; <10% at time points above 6 min) were omitted.

From the data of Table 1, it follows that when compared against A_1, both additional lots of tablets of brand A (A_2 and

Table 1 – Results of the pairwise comparison of eight lots of Furosemide tablets, employing the difference (f_1) and similarity (f_2) criteria[a]

Lot	*f*-Criterion	A_1	A_2	A_3	B_1	B_2	B_3	C_1	C_2
A_1	f_1		2.7	2.8	4.0	3.7	6.5	11.3	8.9
	f_2		78.0	78.4	77.0	70.7	63.5	53.7	59.2
A_2	f_1	2.8		1.0	5.0	6.6	9.4	8.8	6.4
	f_2	78.0		94.5	67.4	64.6	58.2	57.7	64.6
A_3	f_1	2.9	1.0		4.3	5.9	8.7	9.4	7.0
	f_2	78.4	94.5		68.5	67.1	60.1	55.8	61.9
B_1	f_1	4.0	4.8	4.2		4.8	6.1	11.6	9.2
	f_2	77.0	67.4	68.5		74.1	67.4	51.3	56.2
B_2	f_1	3.6	6.2	5.6	4.7		2.6	14.5	12.1
	f_2	70.7	64.6	67.1	74.1		84.1	46.9	51.1
B_3	f_1	6.1	8.6	8.0	5.7	2.6		16.7	14.4
	f_2	63.5	58.2	60.1	67.4	84.1		43.9	47.4
C_1	f_1	12.7	9.7	10.4	13.1	16.9	20.0		2.7
	f_2	53.7	57.7	55.8	51.3	46.9	43.9		81.1
C_2	f_1	9.7	6.8	7.5	10.1	13.8	16.8	2.6	
	f_2	59.2	64.6	61.9	56.2	51.1	47.4	81.1	

[a] Letters designate different brands; numbers differentiate between different lots of the same brand. Non-complying figures are shown in italics.

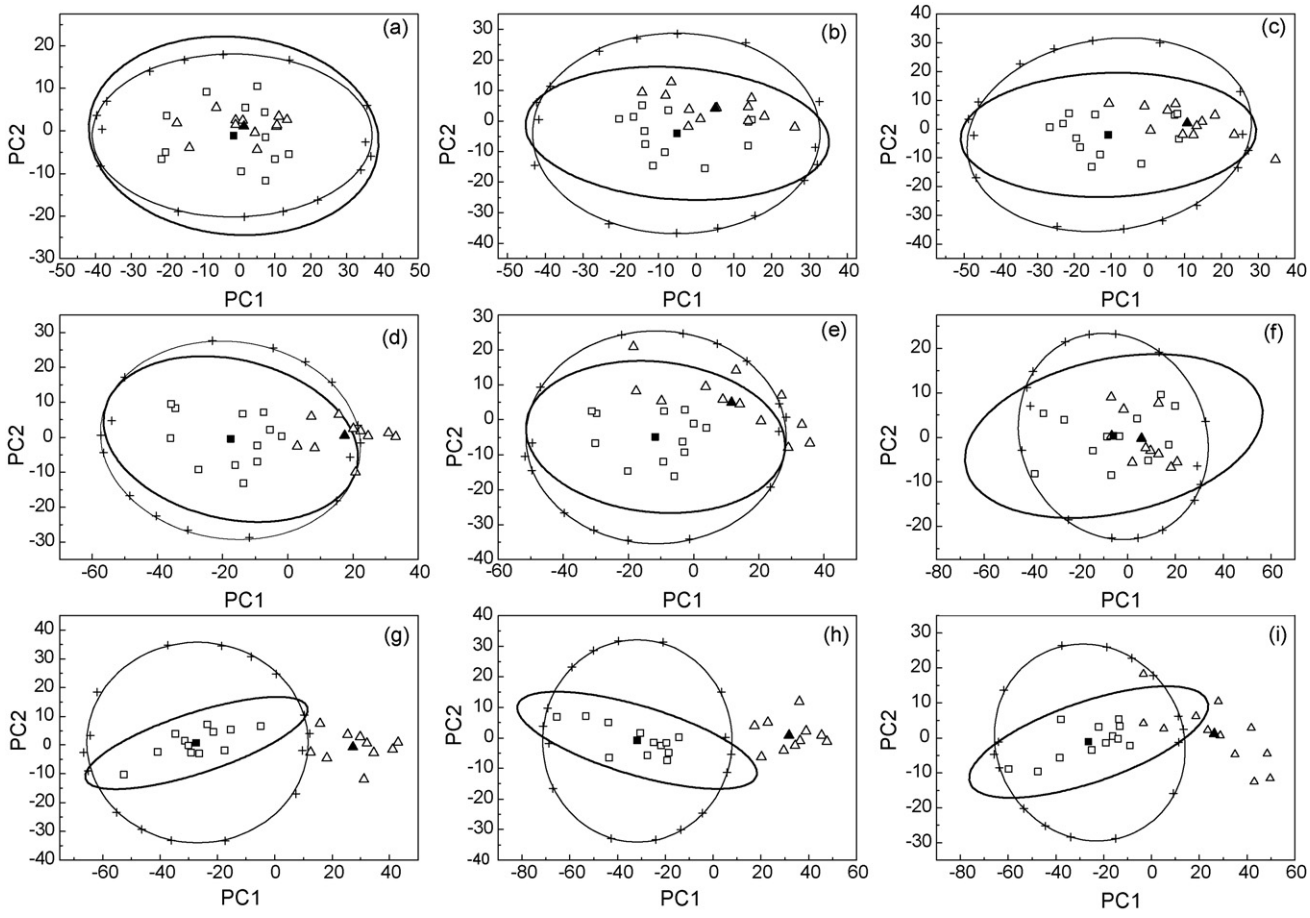

Fig. 2 – Weighed scores plot for the PCA-based pairwise comparison between dissolution data of reference (□) and test (△) lots of Furosemide. (a) A_1–A_2; (b) A_1–B_1; (c) A_1–B_2; (d) A_1–C_1; (e) A_1–C_2; (f) C_1–C_2; (g) B_2–C_1; (h) B_3–C_1; (i) B_3–C_2. The 95% confidence region (—), as well as the $f_2 = 50$ ellipse (–+–) and the means of the PC scores of the reference (■) and test (▲) lots are also shown.

A_3) exhibited acceptable difference (2.7 and 2.8) and similarity (78.0 and 78.4) results; analogously, the parameters for the three lots of brand B ($f_1 = 3.7$–6.5 and $f_2 = 63.5$–77.0) indicated that they should be considered similar to A_1. The f_1/f_2-test also suggested that tablets of brand B were similar among them and with brand A. On the other hand, although complying with the requirements for similarity when compared against the tablets of brand A, brand C exhibited a different range of f_1/f_2 values ($f_1 = 6.4$–11.3 and $f_2 = 53.7$–64.6), which shifted more towards non-compliance when analyzed against the data of lot B_1 ($f_1 = 9.2$–11.2 and $f_2 = 51.3$–56.2). This trend was more clearly evidenced when they were tested against lots B_2 and B_3, furnishing in some cases non-complying values. Interestingly, both lots of brand C demonstrated to be similar to each other ($f_1 = 2.6$, 2.7 and $f_2 = 81.1$). Among the tested lots, both f-factors allowed to arrive at the same conclusion, except in the case of the B_2–C_1 comparison, where the f_1 estimator suggested "similarity", while its f_2 counterpart indicated that the lots were not similar.

To evaluate the performance of the PCA-CR method, Hotelling's test was run and, since no outliers were detected, the weighed scores of the first two PCs of pairs of Furosemide lots were plotted, with selected results shown in Fig. 2. Each plot displays the corresponding 95% confidence ellipse and the coordinates of the mean values of the weighed scores of the reference and test lots. The regions where most of the samples would exhibit $f_2 = 50$, calculated employing the bootstrapping technique, are also included (Shah et al., 1998).

The images clearly show that lots A_2, B_1 and B_2 can be considered similar to A_1 (Fig. 2a–c), despite that one of the samples of lot B_2 falls out of the 95% confidence region (Fig. 2c). On the other hand, and contradicting f_1/f_2 predictions, non-similarity between A_1 and both batches of brand C tablets is evident, despite of the fact that C_1 and C_2 exhibit similarity to each other (Fig. 2f). However, since in the A_1–C_1 comparison only four tablets of the test lot fall outside of the 95% confidence ellipse, should a multiple stage acceptance rule be in practice (Tsong et al., 1995; USP Convention, 2007), lot C_1 could perhaps be considered for a second stage. This instance, while representing a less demanding standard than the single stage PCA-CR method, may still be more discriminant than the f_1/f_2-criteria. As expected, comparison between lots B and C clearly evidenced non-similarity despite that some of the observed f_2 values (>45) were relatively close to the lower acceptable limit.

The similarity and difference factors appear to be simple and easy to be calculated; perhaps this is the key for their

adoption by the industry, despite that they impose restrictions to the quality of the data to be used (not more than one point above 85% dissolution and specific limits to the CV% at different points of the dissolution profile), and their outcome exhibit some dependence upon the number and position of time points employed (Polli et al., 1997). The similarity function f_2 has been severely criticized by several authors (Eaton et al., 2003) arguing that it also lacks statistical justification (Tsong et al., 1996; Shah et al., 1998; Ma et al., 1999; Chow and Shao, 2002), and performs unnecessary use of the logarithmic reciprocal square root transformation, which makes its statistical distribution very complicated and almost intractable (Liu et al., 1997).

Contrarily, the proposed PCA-CR method offers a simple graphical and analytical way to decide about similarity, employing sound mathematical and statistically based procedures. In addition, it is able to make use of all the available data points, regardless the amount of drug dissolved and data variability. This is highly advantageous, since it provides a better appreciation of the dissolution behaviour of the lots being compared.

The PCA-CR results for lots A and B showed good agreement with the outcome of the corresponding determinations of f_1 and f_2; however, both methods provided different conclusions for the comparison between lots A and C. In the *f*-test, brand C exhibited compliance but f_1 (>6.0) and f_2 values (<60) were observed to fall in a different range than those of brands A and B. This borderline compliance of both lots of brand C in the f_2-test and non-compliance with the PCA-CR method reflects the fact that the latter method represents a slightly rigorous standard than the *f*-based approach, being perhaps anticipating non-similarity, as detected when brands B and C were compared.

Both the *f*-based and the PCA-CR methods revealed a closer likelihood of brand C towards brand A than with regards to brand B. In fact, the *f*-based A–C comparison suggested "similarity", while the B–C comparison indicated "non-similarity" in the B_1/B_2–C_1/C_2 cases; analogously, in the case of the PCA-CR counterpart, while concluding for "non-similarity" in every case, fewer points remained outside the 95% confidence region in the A–C comparisons (Fig. 2c and d) than in the B–C comparisons (Fig. 2g–i). On the other hand, as in the *f*-based comparison, both lots C – of analogous shape and size – were considered similar to each other, despite not being able to achieve "similarity" with the A_1 reference lot.

Interestingly, significant correlations were obtained when the number of data points left out of the confidence ellipses were plotted against f_1 or f_2 values. However, despite being correlated to the *f*-factors, the PCA-CR represents a more rigorous standard, being devoid of some of their major drawbacks.

3.3. *Dissolution of Acetaminophen tablets*

Fig. 3a displays the dissolution profiles of three different brands of Acetaminophen tablets, and Table 2 contains the f_1/f_2 values of all possible brand-to-brand comparisons, prepared with data taken at 6, 10, 14, 30 and 45 min.

According to the *f*-criteria, only brands A and B should be considered "similar". After running the outlier detection test and demonstrating the suitability of all the dissolution curves,

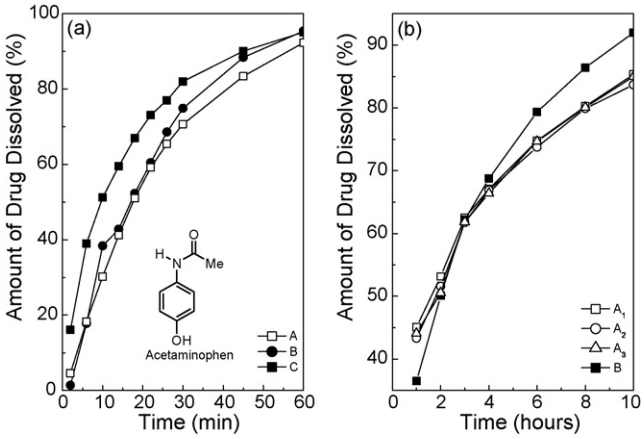

Fig. 3 – (a) Dissolution profiles of three different brands of Acetaminophen tablets. (b) Dissolution profiles of three standard lots ($A_{1–3}$) and a new lot (B). Data were taken from Tsong and Hammerstrom (1994). For the sake of clarity, error bars (<10% at all the time points) were omitted.

it was observed that this result was in perfect agreement with the conclusions emerging from application of the proposed PCA-CR method (Fig. 4a).

Regarding brand C, it exhibited non-complying f_1 and f_2 values, with the data suggesting a possible borderline situation for the B vs. C comparisons ($f_1 = 15.2$ and 17.7; $f_2 = 47.4$). Although the latter seemed amenable for a second stage testing, in case of employing a multiple stage acceptance rule (Tsong et al., 1995; USP Convention, 2007), the PCA-CR weighed scores plot demonstrated beyond doubt that brand C was unable to achieve the "similarity" requirements at this stage (Fig. 4c), not qualifying for further "similarity" testing. The same "non-similarity" conclusion was obtained after comparing brands A and C (Fig. 4b). For the sake of discussion, the B–A comparison is also shown (Fig. 4d); despite that the conclusion about "similarity" agrees with the *f*-based prediction, owing to different data variability within the reference brand (B), the test brand (A) exhibits two borderline dissolution curves.

Interestingly, areas of the 95% confidence ellipses not always were smaller in size than areas enclosed by the $f_2 = 50$ ellipses, calculated under bootstrap assistance. This is because, unlike the f_2 procedure, the size of the confidence ellipses is related to the variability of the data in the reference samples as well as to size and shape of the dissolution curves.

Table 2 – Results of the pairwise f_1/f_2 comparison of three lots of Acetaminophen tablets[a]

Brand	*f*-Criterion	A	B	C
A	f_1		7.4	20.0
	f_2		65.8	43.2
B	f_1	7.9		15.2
	f_2	65.8		47.4
C	f_1	25.0	17.7	
	f_2	43.2	47.4	

[a] Non-complying figures are shown in italics.

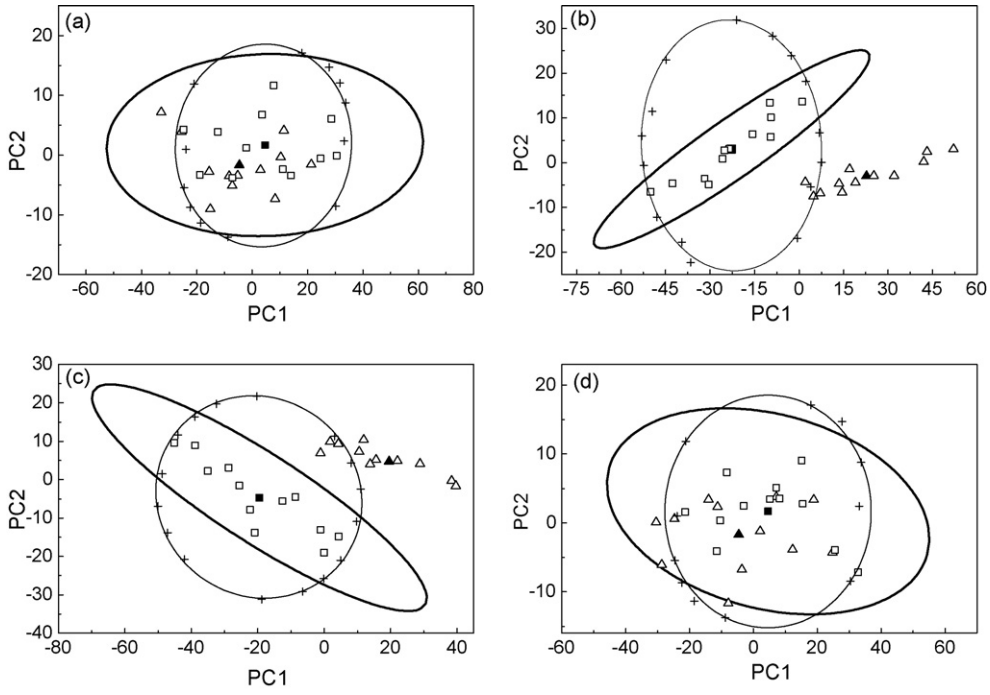

Fig. 4 – Scores plot for PCA-based pairwise comparison between dissolution data of reference (□) and test (Δ) brands of Acetaminophen tablets. (a) A–B; (b) A–C; (c) B–C; (d) B–A. The 95% confidence ellipses (—), the region enclosing acceptable f_2 (≤50) values (–+–) and the means of the PC scores of the reference (■) and test (▲) brands, are also shown.

3.4. *Dissolution data of Tsong and Hammerstrom*

These dissolution data (Fig. 3b) have been previously employed in different cases for dissolution profile comparisons (Chen and Tsong, 1997; Tsong et al., 1997). All of the f_1/f_2 possible pairwise comparisons between the three standard lots (A_1–A_3) and a fourth lot (B) are consigned in Table 3. The results indicate that the lots have "similar" dissolution characteristics, whichever of them is taken as reference. Noticeably, however, the f_2 values for test lot B against the lots A are in a markedly different range (63.5–64.7) from those of the lots A, when tested against each other (90.3–94.6).

No outlier curves were found in the dataset. Plots of the weighed scores of the first and second PCs of the three pre-approved batches (A_1, A_2 and A_3) and test batch B are depicted in Fig. 5. Here, all the PCA-based comparisons of the former demonstrated their similarity, despite that the A_1–A_2 comparison plot exhibited two tablets of the test lot out of the 95% confidence ellipse and those of A_2–A_3 and A_1–A_3 showed one tablet each, out of the confidence region.

On the other hand, pairwise comparison of batches A_1 and A_2 with test batch B, revealed that the latter could not be considered "similar" to any of the former two, surprisingly complying with similarity requirements only with batch A_3, mainly due to its particular data variability. Despite that the f_1/f_2 criteria suggest similarity between all of the dissolution profiles, this is somehow in agreement with conclusions reached by Tsong and co-workers on the grounds of Mahalanobis distance-based multivariate region specification criteria (Chen and Tsong, 1997), and on the basis of confidence intervals of the characteristic parameters (α and β) of a Weibull curve fit (Tsong et al., 1997). The bootstrap-calculated acceptable values of the corresponding f_2 test shown in Fig. 5 reveals that, being of a more permissive nature, the f_2 factor estimation also supports the conclusion of lot similarity.

The graphical result of the A_1–A_2 comparison (and those of A_2–A_3 and A_1–A_3 to a minor extent) can be attributed to higher tablet data variability within the latter lot, compared with the reference. Since the lengths of the axes of the ellipse are related to the eigenvalues of the covariance matrix, the confidence region is sensitive to variability of the reference data; therefore, it should be made possible for some samples of the test lot to remain outside the confidence region due to their own (and sometimes higher) variability, as proposed.

Table 3 – Results of the pairwise comparison of standard lots A_1, A_2 and A_3, and a new lot (B), employing the f_1 and f_2 criteria

Lot	*f*-Criterion	A_1	A_2	A_3	B
A_1	f_1		1.6	1.2	1.5
	f_2		90.3	91.2	63.8
A_2	f_1	1.6		0.4	3.1
	f_2	90.3		94.6	63.5
A_3	f_1	1.2	0.4		2.7
	f_2	91.2	94.6		64.7
B	f_1	1.5	3.0	2.7	
	f_2	63.8	63.5	64.7	

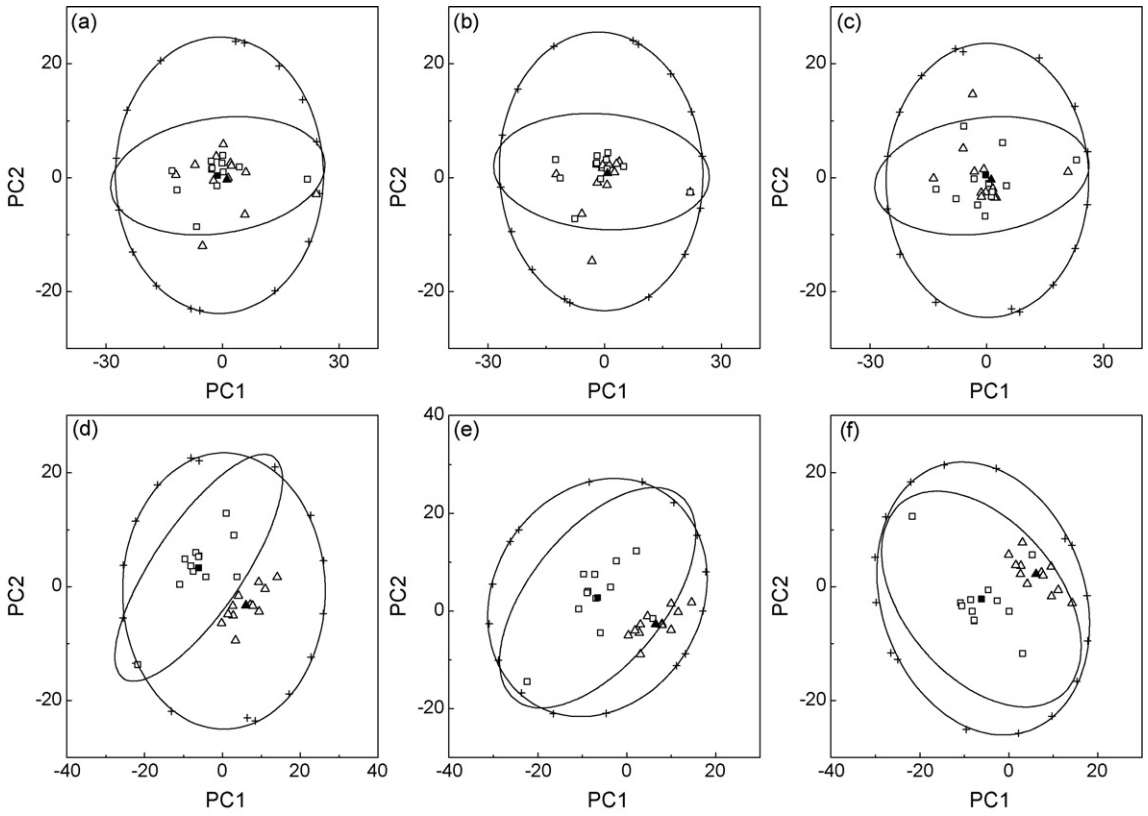

Fig. 5 – PCA-based comparisons [reference (□) and test (△)] between standard batches A_1, A_2 and A_3, as well as with a new test batch (B). Data were taken from Tsong and Hammerstrom (1994). (a) A_1–A_2; (b) A_2–A_3; (c) A_1–A_3; (d) A_1–B; (e) A_2–B; (f) A_3–B. Confidence ellipse of 95% (—), the $f_2 = 50$ ellipse (–+–), and the means of the PCs of the reference (■) and test (▲) lots are also shown.

To take into account data variability is another important feature of the PCA-CR method, in sharp contrast with the f_2 criterion, which is a function of mean differences, and has been criticized for not computing variability within the test and reference data. Not without reason, authors have recommended careful interpretation of f_2 results when the variances of the individual profiles are very different (Saranadasa and Krishnamoorthy, 2005).

Considering that the confidence region in the proposed method represents a tighter standard than the f_1/f_2 indicators, the allowance of up to 20% of the samples to fall outside of the 95% confidence ellipse represents a compromise which transforms the proposed PCA-CR method into a less restrictive tool and a test procedure with pharmaceutical significance, still remaining diagnostic of "similarity".

3.5. *Pre- and post-change dissolution data of Shah et al. (1998)*

The dissolution data of one pre-change and five post-change batches are shown in Fig. 6a, with the f_1/f_2 results of the post-change batches against the pre-change sample consigned in Table 4. Many results (three out of five for f_1 and four out of five for f_2) seem to be borderline ($f_1 > 13$ or $f_2 < 60$). However, despite the differences among the curves and according to the f-criteria, all of the post-change batches comply with the requirements for "similarity".

In their study of this dataset employing bootstrap techniques, Ma et al. concluded that, depending on the estimators employed, only batch B or batches B and E could be considered "similar" to the pre-change batch A (Ma et al., 2000). The PCA-CR analysis of the data was run after assuring absence of outliers. Interestingly, however, this revealed that even batch B does not met the "similarity" requirements, displaying five out of its 12 data points out of the confidence ellipse.

Closer inspection of the dissolution curves of batches A and B indicated that the CV% of the curves of batch B at the different time points (10.6, 9.9, 5.7 and 1.6%) were different from those of the reference batch, being data dispersion of the latter comparatively smaller (6.7, 4.8, 3.8 and 2.9%). Tentatively, this can provide an explanation to the PCA-CR

Table 4 – Comparison of the dissolution profiles of pre-change batch A with five post-change lots, according to the f_1/f_2 criteria

f-Criterion/lot	B	C	D	E	F
f_1	8.8	13.3	13.6	7.4	13.8
f_2	67.1	58.4	58.7	57.5	55.4

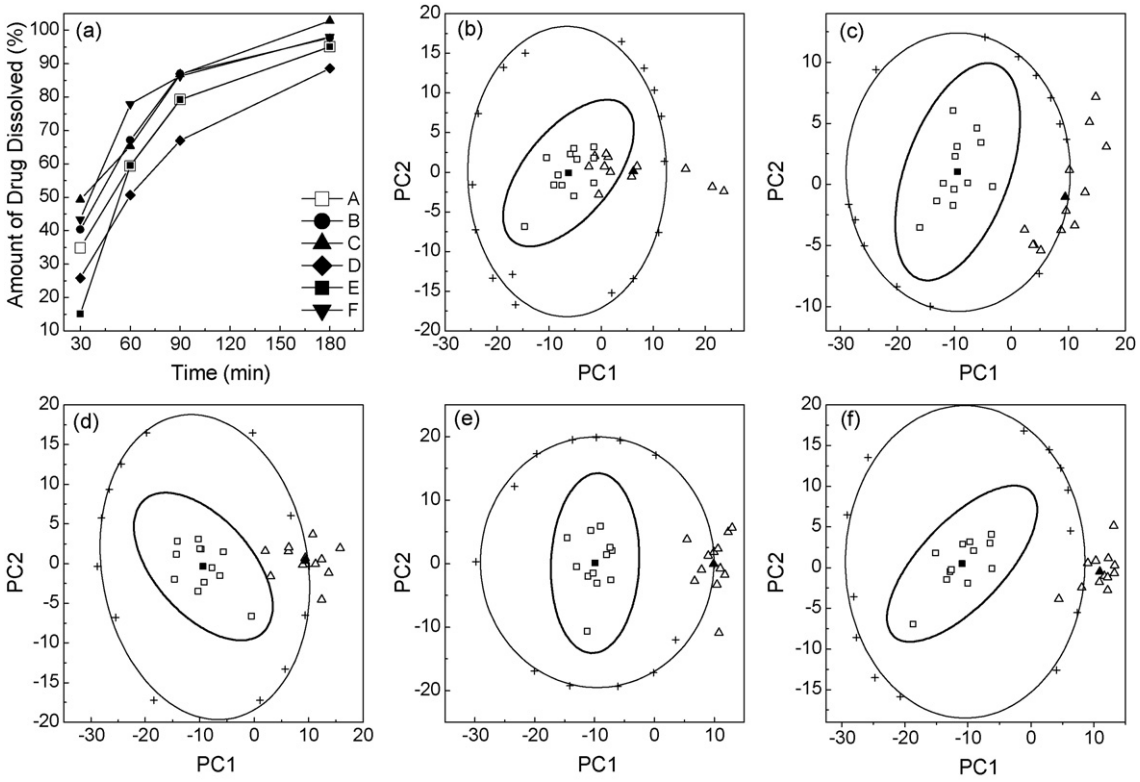

Fig. 6 – (a) Dissolution profiles of (A) pre-change batch; (B) post-change batch 1; (C) post-change batch 2; (D) post-change batch 3; (E) post-change batch 4; (F) post-change batch 5. Data were taken from Shah et al. (1998). (b–f) PCA-based comparisons [reference (□) and test (△)] between pre-change batch A and post-change batches 1–5, respectively. The 95% confidence ellipses (—), the $f_2 = 50$ ellipses (–+–), and the means of the PCs of the reference (■) and test (▲) lots are also shown.

non-similarity result, on account of the sensitivity of the method to data variability, particularly in the reference lot. Indeed, the multivariate test allows to conclude that despite the seemingly analogous shapes of the reference and test profiles, the individual dissolution curves in both batches being compared behave different, hence, "similarity" criteria could not be reached.

When dissolution profiles of pre-change batch A and post-change batch E were compared, it was evident that except for the first time point (where batch E also exhibits considerably less drug dissolved than batch A), both have similar CV% (15.0, 4.8, 3.8 and 2.8% for batch E); however, since PCA-CR is sensitive to shape and size of the dissolution curves (Adams et al., 2001), batch E was also correctly interpreted by the multivariate method as possessing a "non-similar" profile.

3.6. *Method flexibility*

The need for counting at least 80% of the test tablets (Chen and Tsong, 1997) inside of the 95% confidence ellipse of the reference lot constitute arbitrary criteria for assessing "similarity", which relate to the strictness of the proposed method with regards to decision taking. In this sense, PCA-CR represents a more rigorous standard than the *f*-based comparison. However, the proposed approach is flexible enough, so these proposed specifications do not rule out alternative combinations of confidence levels for the ellipses and number of test tablets allowed to remain outside of the confidence region, which might be set according to the experience or specific needs. Boostrap studies are suitable means to provide evidence for this fine-tuning of the method.

4. Conclusions

In summary, the use of the weighed scores plot of the relevant principal components of the dissolution curves with 95% confidence regions (PCA-CR) has been proposed as a new and alternative strategy for the comparison of *in vitro* dissolution profiles of tablet preparations. The results observed with this multivariate approach exhibited good qualitative correlation with the f_1 and f_2 values computed from the dissolution profiles; however, conclusions regarding profile similarity were not always coincident.

This was mainly due to the facts that the proposed method is more discriminating, taking into account data variability within the reference lot in order to build the confidence ellipses. Variations within the test lot, as well as shape and size of the dissolution curves have also influence on the final result.

Unlike the f_1/f_2 methods, based on comparison of data means, the use of confidence ellipses built upon PC values of the individual tablet dissolution curves of the reference set allows a simple and rapid graphic assessment of data distribution. In addition, the proposed approach does not impose

restrictions to useful data in terms of their variability and number of allowed time points above a given degree of dissolution; making use of the all the available information, avoids data-dependent outcomes, a characteristic feature of the *f*-based methods.

Compared to previously reported PCA-based methodologies, the SVD operation carried out on a single matrix containing test and reference data allows the optimization of system parameters in such a way that an improved projection of the test data in the joint reference–test data space is achieved.

The use of boostraping techniques for the representation of the $f_2 = 50$ frontiers in the PCA scores' space, and their comparison with the region enclosed by the 95% confidence ellipses clearly demonstrated the relationship between the official and the proposed methodologies, revealing the potential of PCA-CR under the proposed conditions, as a stricter but still useful tool for providing pharmaceutically sound results in the assessment of "similarity" between different batches of the same product, or products of different brands containing the same active ingredient.

The proposed approach is dependable, it can be easily implemented and profile comparison results are quickly obtained; with minor modifications, it could also be adapted to a multiple stage acceptance rule, as given in the USP.

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